TOTALLY SYNTHETIC ANALOGUES OF SIASTATIN B†

III. TRIFLUOROACETAMIDE ANALOGUES HAVING INHIBITORY ACTIVITY FOR TUMOR METASTASIS

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A trifluoroacetamide analogue of siastatin B, (3*S*,4*S*,5*R*,6*R*)-6-(trifluoroacetamido)-4,5-dihydroxy-3-piperidinecarboxylic acid has been chemically synthesized. This compound, as well as the previously synthesized analogue, (3*R*,4*R*,5*R*,6*R*)-6-(trifluoroacetamido)-3,4,5-trihydroxy-3-piperidinecarboxylic acid, showed marked inhibitory activity against β-glucuronidase and significant inhibition of experimental pulmonary metastasis of the highly metastatic melanoma B16.

Many naturally occurring and synthetic azasugars are potent and specific inhibitors for enzymes associated with carbohydrate metabolism, and they have the potential to produce a number of kinds of beneficial therapeutic effects such as antihyperglycemic, antimetastatic, antifungal, and antiviral activities, *etc.*[1]. Recent studies[2~8] have provided considerable evidence of increased levels of β-glucuronidase activity in human tumors and suggested that β-glucuronidase may play a role in the metastasis of tumor cells.

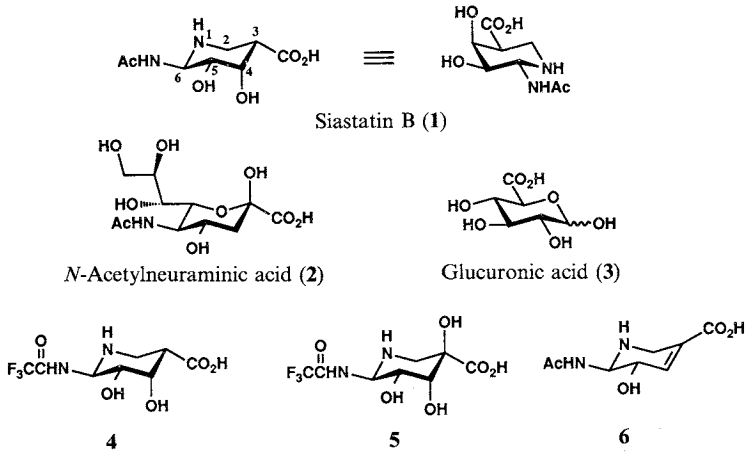
A multifunctional piperidine, siastatin B (**1**) which was isolated as an inhibitor of neuraminidase and β-glucuronidase by UMEZAWA *et al.*[9] from a *Streptomyces* culture, resembles structurally sialic acid (*N*-acetylneuraminic acid, **2**) and glucuronic acid (**3**). After achievement of the total synthesis[10~12] of **1**, we synthesized several branched-chain[13,14] and chemically modified analogues[15~19]. Now, we have synthesized a trifluoroacetamide analogue of siastatin B, (3*S*,4*S*,5*R*,6*R*)-6-(trifluoroacetamido)-4,5-dihydroxy-3-piperidinecarboxylic acid (**4**) (Fig. 1). In the course of our study to investigate the relationships between structure and biological activity of analogues of **1**, it was shown that the 3-hydroxy analogue **5** was a strong inhibitor[14] of β-glucuronidase and the 3,4-olefin analogue **6**[15,18] weakly affected β-glucuronidase. This led to the synthesis of **4** having no hydroxyl group at C-3, which resembles **3**. Compound **4** and the previously synthesized analogue of **1**, (3*R*,4*R*,5*R*,6*R*)-6-(trifluoroacetamido)-3,4,5-trihydroxy-3-piperidinecarboxylic acid (**5**)[14], showed inhibitory effects of experimental pulmonary metastasis of B16 melanoma.

Synthesis

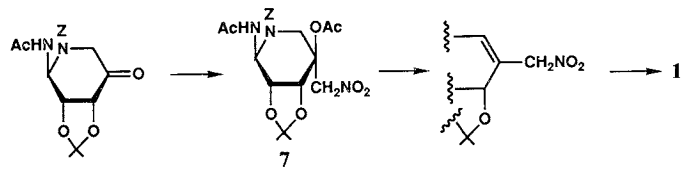
In the total synthesis[10~12] of **1**, the stereospecific introduction of the carboxyl group was carried out *via* endocyclic nitro olefin obtained by base-catalyzed elimination of the acetoxy group of **7**, as shown in

† The correct clockwise numbering is employed for siastatin B and its analogues in this article according to IUPAC rules.

Fig. 1. Structure of siastatin B, siastatin B analogues, *N*-acetylneuraminic acid and glucuronic acid.



Scheme 1.



Scheme 1. In this synthesis, however, the trifluoroacetamide group in **8** was unstable under the conditions of base-catalyzed elimination. Thus, as shown in Scheme 2, we developed an alternative using the Wacker process[20] oxidation of the enol ethers **10** and **11** prepared by the one-carbon homologation of the ketone **9** using the Witting reaction. The starting (3*S*,4*S*,5*R*,6*S*)-1-(*tert*-butoxycarbonyl)-6-(trifluoroacetamido)-4,5-(isopropylidenedioxy)-3-piperidinol (**8**) obtained by the method described previously[14], was oxidized with ruthenium tetraoxide to give **9** in a good yield. Reaction of **9** in tetrahydrofuran (THF) with an excess of a solution of (benzyloxymethylene)triphenylphosphorane generated from (benzyloxymethyl)triphenylphosphonium chloride and phenyllithium in THF afforded (*Z*)-benzyloxy ether **10** and (*E*)-isomer **11** in yields of 26 and 23%, respectively. The stereochemistry around the double bonds in **10** and **11** was tentatively determined by NOE experiments. NOE was observed between the olefin proton and the equatorial proton at C-2 in **10**, while no such effect was observed in **11**. Initial attempts to convert **10** and **11** into the corresponding hydroxymethyl derivatives directly by catalytic hydrogenolysis[21] utilizing palladium or Raney Ni were unsuccessful. However, oxidation of **10** and **11** by the Wacker process using palladium chloride and copper (I) chloride in *N*,*N*-dimethylformamide-water (10:1) gave the ester **12**. Fig. 2 shows a possible reaction mechanism[22] *via* the Wacker process. The boat conformations in **10** and **11** should be predictable from the boat conformation of synthetic (2*R*,3*S*,4*S*,5*R*)-6-acetamido-1-(*tert*-butoxycarbonyl)-4,5-(isopropylidenedioxy)-3-(nitromethyl)-3-piperidinol (**13**) determined previously by X-ray crystallographic analysis[13]. Small coupling constants ($J = < 2$ Hz) between 5-H and 6-H in the ^{1}H NMR spectra of **10** and **11** in agreement with that of **13** also support the boat conformations of **10** and **11**. The π-complex **14** is formed by attack of the palladium reagent from the less-hindered side. The

Scheme 2.

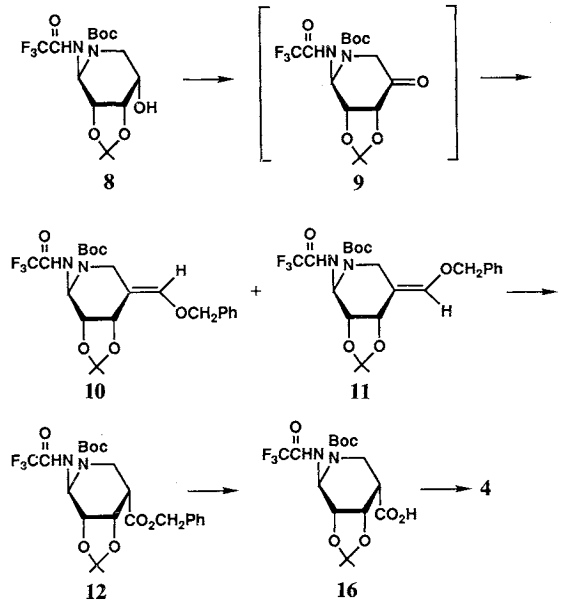
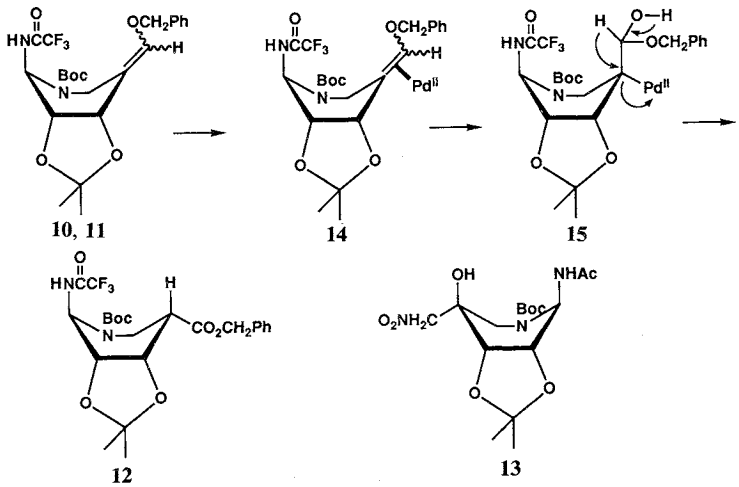


Fig. 2. A possible reaction mechanism *via* the Wacker process.



unstable σ-alkyl intermediate **15**, formed by subsequent addition of water to the double bond, is transformed into the benzyl ester **12** by a 1,2-hydride shift and reductive elimination of the palladium. Catalytic hydrogenolysis of **12** with palladium on carbon afforded **16**, which was converted into **4** by treatment with hydrogen chloride in 1,4-dioxane in a good yield.

Biological Activities

As shown in Table 1, **4** inhibited β-glucuronidase from bovine liver as strongly as **5**. Compound **4**, as well as **5**, also showed weak inhibitory activity against α-glucosidase (yeast) and did not inhibit sialidases isolated from *Streptococcus* sp. and the A/Aichi/2/68 (H3N2) strain of influenza virus. On the other hand,

Table 1. Inhibition (%) at 100 μg/ml against glycosidases.

Compound	α-Glucosidase (yeast)	β-Glucosidase (almond)	α-Mannosidase (soybean)	β-Glucuronidase (bovine liver)	*N*-Acetylneuraminidase	
					Streptococccus	Influenza virus A Aichi/2/68 (H3N2)
1	3	24	2	85 (15.5)	41 (6.3)	20
4	70 (40)	85 (19)	4	100 (0.008)	0	2
5	87 (7.7)	22	7	100 (0.02)	2	15
6	88 (16)	4	0	81 (22.5)	70 (3.1)	39

( ): IC_{50}, μg/ml.

6 showed weak inhibition of β-glucuronidase and α-glucosidase, and affected moderately *Streptococcus* sialidase. Lung colonization after intravenous transplantation of the highly metastatic B16 cells isolated by FIDLER's[23] modified method[24] was suppressed dose-dependently by *in vitro* pretreatment with **4** and **5** as shown in Table 2, while **6** did not suppress the lung colonization. As shown in Fig. 1, **4** and **5** have the same topographical orientation of the functional groups as glucuronic acid (**3**). Compounds **4** and **5** probably mimic glucuronic acid in ground-state binding to β-glucuronidase and strongly inhibit the enzymatic reaction. Recent studies by NAKAJIMA *et al.*[2,6,25,26] proved that heparanase (endo-β-glucuronidase) activity correlates with the lung colonization abilities of murine B16 melanoma cells by extracellular matrix degradation and is inhibited by heparanase inhibitors such as heparin, heparin derivatives, *etc.* It is suggested that **4** and **5** as glucuronidase inhibitors inhibit extracellular matrix degradation and/or modify cell-surface glycoconjugates of B16 melanoma cells, resulting in the inhibition observed of experimental pulmonary metastasis of the highly metastatic B16 line. Futher evaluation of the biological activities of these compounds is under investigation.

Table 2. Effect on the experimental metastasis of the highly metastatic melanoma B16 cells in mice.

Compound	Concentration (μg/ml)	Inhibition (%)
None		0
4	10	48.5
	30	61.9
	50	90.8
5	10	11.9
	30	75.0
	50	80.5
	100	90.4
6	10	27.4
	30	35.4

Experimental

General Methods

Melting points were determined with a Yanagimoto apparatus and were uncorrected. IR spectra were determined on a Hitachi Model 260-10 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. ^1H NMR spectra were recorded with a JEOL JNM EX270 spectrometer. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane as an internal standard. Ms spectra were taken by a JEOL JMS-SX102 in the FAB mode.

Enzyme Inhibition Assay

α-Glucosidase (yeast)[27], β-glucosidase (almond)[28], α-mannosidase (soybean)[29] and β-glucuronidase (bovine liver)[30] assays were evaluated by methods described in references by Dr. S. OHUCHI (Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd.). *N*-Acetylneuraminidase assays of *Streptococcus*[18] and influenza virus A Aichi/2/68 (H3N2)[9] were evaluated by methods described in references by Dr. I. KIJIMA-SUDA (Central Research Institute, MECT Corp.) and by Prof. T. AOYAGI and Ms. S. HARADA (Institute of

Microbial Chemistry), respectively.

Experimental Metastasis Assay[23,24]

The highly metastatic melanoma B16 cells (3×10^5 cells) were cultured in DULBECCO's modified EAGLE's medium supplemented with fetal bovine serum under 5% CO_2 at 37°C for 24 hours. Cells were incubated with (or without) each test compound under the same condition for 72 hours. After treatment with 0.05% trypsin and 0.02% EDTA solution, a cell suspension containing 1×10^6 cells in 1 ml of divalent cation-free DULBECCO's phosphate-buffered saline was prepared. Cell (1×10^5 in 0.1 ml) were injected intravenously into the tail vein of each mouse (male BDF_1, 7 weeks old). Fourteen days later, after tumor cell implantation, the mice were autopsied. The number of pulmonary tumor nodules was counted. Inhibition (%) of metastasis was calculated from the ratio of tumor nodules in treated and control experiments.

(4*S*,5*R*,6*S*)-3-[(*Z*)-Benzyloxymethylene]-1-(*tert*-butoxycarbonyl)-6-(trifluoroacetamido)-4,5-(isopropylidenedioxy)piperidine (**10**) and (4*S*,5*R*,6*S*)-3-[(*E*)-Benzyloxymethylene]-1-(*tert*-butoxycarbonyl)-6-(trifluoroacetamido)-4,5-(isopropylidenedioxy)piperidine (**11**)

A solution of RuO_4 in CCl_4 prepared from RuO_2 (260 mg) and $NaIO_4$ (400 mg) in a mixture of H_2O (38 ml) and CCl_4 (38 ml) was added to a solution of **8**[14] (140 mg) in CH_2Cl_2 (5 ml) until the appearance of a yellow color, and the mixture was stirred at room temperature for 15 minutes. After being quenched with 2-propanol (4 ml), the mixture was diluted with CH_2Cl_2. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil. The resulting oil was subjected to preparative TLC on silica gel, developing with $CHCl_3$-MeOH (20:1), to give **9** (114 mg) as an amorphous solid. To a cooled (−68°C), stirred solution of (benzyloxymethyl)triphenylphosphonium chloride (1.82 g) in THF (3.5 ml) under Ar was added dropwise a 1.8 M solution of phenyllithium in hexane-ether (7:3) (2.15 ml), and the mixture was stirred for 10 minutes. To the resulting solution of (benzyloxymethylene)-triphenylphosphorane was added dropwise a solution of **9** (114 mg) in THF (1 ml) at −68°C, and the mixture was allowed to warm to 10°C with continuous stirring during 4.5 hours. After being quenched with saturated NH_4Cl solution, the aqueous mixture was extracted with CH_2Cl_2. The extract was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil. The oil was subjected to preparative TLC on silica gel, developing with toluene-acetone (5:1), to give **10** (37 mg, 25.5%) and **11** (33 mg, 22.8%) as colorless foams.

10: $[\alpha]_D^{26}$ +13° (*c* 0.31, MeOH); IR ($CHCl_3$) 3450, 3000, 2950, 1735, 1700, 1680, 1530, 1470, 1405, 1395, 1390, 1305, 1270, 1170, 1090, 970, 870 cm^{-1}; ^1H NMR (CD_3OD, 40°C) δ 1.35 and 1.40 (each 3H, s, isopropylidene), 1.46 (9H, s, NCOOC $(CH_3)_3$), 3.66 (1H, d, J=13.2 Hz, 2-H_{eq}), 4.02 (1H, dd, J=2.0 and 13.2 Hz, 2-H_{ax}), 4.44 (1H, dd, J=2.0 and 7.6 Hz, 5-H), 4.90 (2H, s, $-OCH_2-$), 5.35 (1H, d, J=7.6 Hz, 4-H), 5.80 (1H, d, J=~2 Hz, 6-H), 6.55 (1H, d, J=2.0 Hz, 7-H) and 7.30~7.37 (5H, m, C_6H_5); FAB-MS (positive) *m/z* 509 $(M+Na)^+$, 487 $(M+H)^+$, 429, 373, 318, 260, 216, 91 and 57.

11: $[\alpha]_D^{26}$ +50° (*c* 0.67, MeOH); IR ($CDCl_3$) 3425, 3025, 2980, 2940, 2900, 1735, 1700, 1520, 1460, 1395, 1390, 1370, 1305, 1250, 1170, 1080, 1020, 970, 950, 920, 870 cm^{-1}; ^1H NMR (CD_3OD, 40°C) δ 1.32 and 1.37 (each 3H, s, isopropylidene), 1.48 (9H, s, $NCOOC(CH_3)_3$), 4.01 (1H, dd, J=~2 and 14.8 Hz, 2-H), 4.08 (1H, dd, J=1.7 and 14.8 Hz, 2-H), 4.37 (1H, dd, J=1.7 and 7.6 Hz, 5-H), 4.67 (1H, d, J=7.6 Hz, 4-H), 4.90, 4.97 (2H, ABq, J=12.5 Hz, $-OCH_2-$), 5.78 (1H, br s, 6-H), 6.56 (1H, dd, J=1.7 and ~2 Hz, 7-H) and 7.30~7.40 (5H, m, C_6H_5); FAB-MS (positive) *m/z* 487 $(M+H)^+$, 431, 373, 318, 260, 216, 91 and 57.

(3*S*,4*S*,5*R*,6*S*)-3-(Benzyloxycarbonyl)-1-(*tert*-butoxycarbonyl)-6-(trifluoroacetamido)-4,5-(isopropylidenedioxy)piperidine (**12**)

A) From **11**: A mixture of $PdCl_2$ (10.2 mg) and CuCl (46.2 mg) in DMF-H_2O (10:1, 0.3 ml) was stirred at room temperature for 1 hour under oxygen atmosphere, then **11** (29.5 mg) was added. The mixture was stirred at 70°C for 25 hours and furthermore at room temperature for 61 hours, and the insoluble material was removed by filtration. Evaporation of the filtrate gave an oil, which was dissolved in EtOAc. The solution was washed with saturated aqueous $NaHCO_3$ and H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil. The oil was subjected to preparative TLC on silica gel, developing with toluene-acetone (5:1) to give **12** (9.5 mg, 31%) as a colorless foam: $[\alpha]_D^{26}$ +13° (*c* 0.42, MeOH); IR ($CHCl_3$) 3450, 3060, 3020, 2970, 1750, 1720, 1560, 1530, 1490, 1470, 1410, 1400, 1395, 1390, 1370, 1330,

1275, 1190, 1090, 1020, 970, 940, 890 cm^{-1}; ^1H NMR (CD_3OD, 40°C) δ 1.33 and 1.40 (each 3H, s, isopropylidene), 1.46 (9H, s, $NCOOC(CH_3)_3$), 3.21 (1H, ddd, J=3.0, 4.6 and 12.5 Hz, 3-H), 3.43 (1H, t, J=12.5 Hz, 2-H_{ax}), 3.70 (1H, ddd, J=1.0, 4.6 and 12.5 Hz, 2-H_{eq}), 4.53 (1H, dd, J=2 and 7.6 Hz, 5-H), 4.85 (1H, ddd, J=1.0, 3.0 and 7.6 Hz, 4-H), 5.21 (2H, s, $-CO_2H_2-$), 5.82 (1H, d, J=2.0 Hz, 6-H) and 7.30~7.40 (5H, m, C_6H_5); FAB-MS (positive) m/z 525 $(M+Na)^+$, 503 $(M+H)^+$, 447, 401, 334, 290, 232, 136, 91 and 57.

B) From **10**: Compound **12** was also obtained from **10** (yield 15%) by a similar procedure as was used for the preparation from **11**.

(3*S*,4*S*,5*R*,6*S*)-1-(*tert*-Butoxycarbonyl)-6-(trifluoroactamido)-4,5-(isopropylidenedioxy)-3-piperidinecarboxylic Acid (**16**)

A solution of **12** (15.4 mg) in EtOAc (1.6 ml) was stirred with 10% palladium on carbon (8 mg) under hydrogen stream for 2 hours. Catalysts were removed by filtration, and the residue was washed with EtOAc. The filtrate and washings were combined and evaporated to give a solid. The solid was subjected to preparative TLC on silica gel, developing with $CHCl_3$-MeOH (5:1), to give **16** (11.6 mg, 91.8%) as a colorless amorphous solid: mp >200°C (dec); $[\alpha]_D^{23}$ +28° (c 0.24, MeOH); IR ($CHCl_3$) 3470, 3380, 3020, 2975, 1735, 1700 (sh), 1695, 1610, 1585, 1490, 1475, 1420, 1410, 1390, 1370, 1340, 1275, 1190, 1090, 1070, 970, 890 cm^{-1}; ^1H NMR (CD_3OD, 40°C) δ 1.33 and 1.39 (each 3H, s, isopropylidene), 1.47 (9H, s, $COOC(CH_3)_3$), 2.91 (1H, ddd, J=2.4, 5 and 12.5 Hz, 3-H), 3.44 (1H, t, J=12.5 Hz, 2-H_{ax}), 3.64 (1H, dd, J=5 and 12.5 Hz, 2-H_{eq}), 4.49 (1H, dd, J=2 and 7.6 Hz, 5-H), 4.84 (1H, dd, J=2.4 and 7.6 Hz, 4-H) and 5.78 (1H, d, J=2 Hz, 6-H); FAB-MS (positive) m/z 435 $(M+Na)^+$, 413 $(M+H)^+$, 379, 329, 222, 176, 136 and 57.

(3*S*,4*S*,5*R*,6*R*)-6-(Trifluoroacetamido)-4,5-dihydroxy-3-piperidinecarboxylic Acid (**4**)

Compound **16** (16.8 mg) was dissolved in 4 M hydrogen chloride in dioxane (0.33 ml), and the mixture was stirred at room temperature overnight. Another portion of 4 M hydrogen chloride in dioxane (0.17 ml) was added to the mixture and then the reaction mixture was further stirred at room temperature for 1.5 hours. The resulting precipitates were collected by filtration and washed with dioxane to give a colorless amorphous solid of **4** as its hydrochloride (12.1 mg, 96.2%): mp 130°C (dec); $[\alpha]_D^{31}$ +27° (c 0.22, H_2O); IR (KBr) 3425, 2950, 2830, 2780, 2490, 1740, 1725, 1715, 1630, 1555, 1465, 1440, 1410, 1360, 1330, 1310, 1280, 1230 (sh), 1210, 1200, 1180, 1160, 1095, 1030, 1010, 970, 940, 915, 870, 840 cm^{-1}; ^1H NMR (D_2O) δ 2.92 (1H, ddd, J=2.3, 8 and 10 Hz, 3-H), 3.32~3.39 (2H, m, 2-H_2), 3.96 (1H, dd, J=2.6 and 10.6 Hz, 5-H), 4.42 (1H, t, J=2.3 Hz, 4-H) and 5.01 (1H, d, J=10.6 Hz, 6-H); FAB-MS (positive) m/z 273 $(M+H)^+$, 207, 160, 141, 115, 75 and 57.

Acknowledgments

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